Claims

- 1. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free, protein obtainable from a peripheral membrane fraction of oviductal apical plasma membrane (APM), or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10 kDa and 100 kDa.
- 2. A method according to claim 1 in which the spermatozoa are contacted with the protein *in vitro*.
- 3. A method according to claim 1 in which the spermatozoa are boar spermatozoa and the peripheral membrane fraction is of porcine oviductal APM.
- 4. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 95 kDa.
- 5. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of from approximately 60 to approximately 70 kDa.
- 6. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated,

cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 70 kDa.

- 7. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 41 kDa.
- 8. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 38 kDa.
- 9. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 37 kDa.
- 10. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of from approximately 19 to approximately 21 kDa.
- 11. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane

fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 19 kDa.

- 12. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 18 kDa.
- 13. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 13.5 kDa.
- 14. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, in which the isolated protein comprises ribonucleotideprotein-2.
- 15. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with ribonucleotideprotein-2 or a fragment or derivative therefrom.
- 16. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with a protein other than ribonucleotideprotein-2 (acidic ribosomal protein P2), which protein includes all or part of the N-terminal sequence derived from acidic ribosomal protein P2.

- 17. A method according to claim 16 wherein said protein includes all or part of the sequence MRYVASYLLA or an analog or homolog thereof.
- 18. A method of improving and/or prolonging sperm viability following cryopreservation which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane protein fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa.
- 19. A method of improving and/or prolonging sperm viability during cryopreservation which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane protein fraction of oviductal APM, or a fragment or derivative therefrom, the isolated protein having a molecular weight of between approximately 10kDa and 100kDa.
- 20. A method of isolating a protein having a molecular weight of between approximately 10kDa and 100kDa, or a fragment or derivative therefrom, having sperm viability improving and/or prolonging activity from oviductal APM comprising the steps of:
- (i) harvesting mammalian oviduct epithelial cells;
- (ii) separation and isolation of a plasma membrane preparation using a magnesium chloride solution, and centrifugation to obtain a crude APM fraction;
- (iii) extraction of a soluble fraction from the crude APM
 fraction using a salt solution and centrifugation of the
 solution obtained;
- (iv) concentration of the supernatant and washing, to obtain protein.

- 21. A method according to claim 16 in which the salt solution used in step (iii) is sodium chloride solution.
- 22. An isolated, cell-free protein having a molecular weight of between approximately 10kDa and 100kDa or a fragment or derivative therefrom, having sperm viability improving and/or prolonging activity, the protein, fragment or derivative obtainable according to the following method:
- (i) harvesting mammalian oviduct epithelial cells;
- (ii) separation and isolation of a plasma membrane preparation using a magnesium chloride solution, and centrifugation to obtain a crude APM fraction;
- (iii) extraction of a soluble fraction from the crude APM fraction using a salt solution and centrifugation of the solution obtained;
- (iv) concentration of the supernatant and washing, to obtain protein.
- 23. A method according to claim 22 in which the salt solution used in step (iii) is sodium chloride solution.
- 24. A method of improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa, in which the spermatozoa are microencapsulated.
- 25. A method according to claim 21 in which the treated spermatozoa are microencapsulated in a semi-permeable membrane comprising poly-lysine.
- 26. A method of improving and/or prolonging sperm viability comprising contacting spermatozoa with an isolated, cell-

free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa, in which the proteins are linked to inert polymers.

- 27. A method according to claim 26 in which hydrophilic polymers are used.
- 28. A method according to claim 26 in which the polymer is amine- and carbonyl-reactive dextran.
- 29. A method for improving and/or prolonging sperm viability which comprises contacting spermatozoa with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa, in which the protein is present in a concentration of between approximately 0.1µg/L and approximately 1g/L.
- 30. A method according to claim 29 in which the protein is present in a concentration of between approximately $5\mu g/L$ and approximately $400\mu g/L$.
- 31. A method according to claim 29 in which the protein is present in a concentration of between approximately $25\mu g/L$ and approximately $200\mu g/L$.
- 32. A method of improving and/or prolonging semen survival following sex-sorting of the spermatozoa for X- (female) and Y-bearing (male) spermatozoa cells which comprises contacting spermatozoa with an isolated protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa.

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of approximately 70kDa, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.

- 39. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 41kDa, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.
- 40. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 38kDa, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.
- 41. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 37kDa, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.
- 42. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of from approximately 19 to approximately 21 kDa, in which the protein, fragment or derivative has sperm viability improving and/or prolonging activity.
- 43. An isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of approximately 19 kDa, in which the protein, fragment or

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derivative has sperm viability improving and/or prolonging
                     activity.
                  An isolated, cell-free protein obtainable from a peripheral
              44.
                  Membrane fraction of oviductal APM, or a fragment or
                derivative therefrom, the protein having a molecular weight
                of approximately 18 kDa, in which the protein, fragment or
              of approximately is koa, in which the protein, tragment or prolonging
              activity.
           An isolated, cell-free protein obtainable from a peripheral
           membrane fraction of Oviductal APM, or a fragment or
          membrane

derivative therefrom, the protein having a molecular weight
         of approximately 13.5 kDa, in which the protein, fragment
        or derivative has sperm viability improving and/or
       prolonging activity.
     An isolated, cell free protein, other than
    ribonucleotideprotein-2 (acidic ribosomal Protein P2),
   which protein includes all or part of the N-terminal
  sequence derived from acidic ribosomal protein P2.
An isolated, cell free protein according to claim 46 which
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includes all or part of the sequence MRYVASYLLA or an analog or h_{Omolog} thereof. Use of an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a and inn fragment or derivative therefrom, the protein naving a common sition for improving and lookba, in the manufacture of a composition for improving and/or prolonging sperm viability. *9*9.

Use of tibonucleotideprotein-2 or a fragment or derivative therefrom, in the manufacture of a composition for improving and/or prolonging sperm Viability. $V:|CNORTH|S_{pec_S|45304}US_{02}|_{09.07}$

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- 50. Use of a protein other than ribonucleotideprotein-2 (acidic ribosomal protein P2), which protein includes all or part of the N-terminal sequence derived from acidic ribosomal protein P2, in the manufacture of a composition for improving and/or prolonging sperm viability.
- 51. Use of a protein which includes all or part of the sequence MRYVASYLLA or an analog or homolog thereof, in the manufacture of a composition for improving and/or prolonging sperm viability.
- 52. Use of an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa, in the manufacture of a composition for improving and/or prolonging sperm viability following cryopreservation.
- 53. Use of an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa, in the manufacture of a composition for improving and/or prolonging sperm viability during cryopreservation.
- 54. Spermatozoa together with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa and having sperm viability improving and/or prolonging activity, which are microencapsulated with a semi-permeable membrane.
- 55. Spermatozoa together with an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal

APM, or a fragment or derivative therefrom, the protein comprising ribonucleotideprotein-2, or a protein other than ribonucleotideprotein-2 (acidic ribosomal protein P2), which protein includes all or part of the N-terminal sequence derived from acidic ribosomal protein P2, or a protein which includes all or part of the sequence MRYVASYLLA or an analog or homolog thereof, and having sperm viability improving and/or prolonging activity, which are microencapsulated with a semi-permeable membrane.

- 56. A method for identifying an isolated, cell-free protein obtainable from a peripheral membrane fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa and having sperm viability improving and/or prolonging activity the method comprising:
- (i) labelling peripheral APM proteins with a marker
- (ii) allowing the labelled APM proteins to bind to surface proteins of spermatozoa
- (iii) washing to remove excess APM

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- (iv) adding a detergent to solubilise sperm surface proteins
- (v) identifying the isolated proteins labelled with the marker which have bound to the surface proteins of the spermatozoa.
- 57. An isolated, cell-free protein obtainable from a peripheral membrane protein fraction of oviductal APM, or a fragment or derivative therefrom, the protein having a molecular weight of between approximately 10kDa and 100kDa and having sperm viability improving and/or prolonging activity, the protein obtained according to the following method:
- (i) labelling peripheral APM proteins with a marker
- (ii) allowing the labelled APM proteins to bind to surface proteins of spermatozoa

(iii) washing to remove excess APM

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- (iv) adding a detergent to solubilise the sperm surface proteins
- (v) identifying the APM proteins labelled with the marker which have bound to the surface proteins of the spermatozoa
- (vi) separating the labelled APM proteins from the surface proteins of the spermatozoa to obtain isolated protein.